Preparation Of Ginkgo Phytosomes By Rotary Evaporation Technique And Its Evaluation

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Abstract:

Phytoconstituents have low permeability, solubility compared to that of synthetic drugs. The objective of the present study is to improve permeability, solubility of phytoconstituents of Ginkgo by combining them with phospholipids and preparing it as phytosomes. Ginkgo biloba is natural drug which contains has two active components. Terpene lactones (ginkgolides and diterpenes) and flavone glycosides (ginkgetin, bilobetin, and sciadopitysin) [1] are the major active chemical constituents. The Ginkgo phytosomes were formulated by using rotary evaporator method. The formulations of phytosomes were analyzed for scanning electron microscopic analysis, particle size, zeta potential, drug content, drug entrapment efficiency, percentage yield and in-vitro drug release. The Release kinetics of Ginkgo phytosome complex[2] was studied using kinetic models. The Ginkgo phytosome formulation containing drug to phospholipid ratio of 1:2, i.e., Formulation F-4 exhibited greater practical yield, drug content, drug entrapment efficiency, less particle size & higher zeta potential and best in-vitro drug release of drug. Formulation F-4 had shown enhanced solubility and better compatibility with the excipients. Therefore it is concluded that Ginkgo phytosomes have good permeability to cross lipid-rich biological membranes and better solubility than that of the Ginkgo drug alone. Phytosomes are able easily compared to drug alone

Keywords - Ginkgo, Ginkgo phytosome complex, Soy lecithin.

DOI Number: 10.14704/nq.2022.20.9.NQ440052 **Neuro Quantology 2022; 20(9):492-499**

1. INTRODUCTION

Phytoconstituents have capability to treat diseases from ancient times. Phyto preparations are used in recent days as they have fewer side effects than synthetic drugs. Plant products are generally polar in nature, i.e., water- soluble. The low biovailabilty of phytoconstituents is due to their large particle size, low permeability through lipid biological membranes, poor absorption and low solubility in biological fluids.

Phytosomes are cell like structures, where “Phyto” means "plants"and "some" means "cell-like" structure[3]. These are recently developed preparations in which water-soluble polar phyto constituents are combined with phospholipids to produce lipid compatible molecular complexes. Phytosomes were developed by mixing of stoichiometric amount of the phospholipid to pure plant constituents in an aprotic solvent. Traditional medications have lower absorption and bioavailability than phytosomes.

Ginkgo biloba is prominent herb in Chinese traditional medicine, a natural herb belonging to Ginkoaceae family. It has potent central nervous system action[4], including improvement of memory, concentration, mental alertness, and lowering of mental exhaustion.

The Action of drug in the central nervous system is because of its antioxidant properties. Ginkgo contains free radical scavengers. It suppresses lipid peroxidation, hence helps to maintain the integrity, permeability of cell walls and protects the neurons from oxidative stress. Free radicals are responsible for Ischemic injury. Ginkgo protects the brain by scavenging reactive oxygen species and free radicals. The antioxidant properties[5] of Ginkgo biloba helps to boost levels of free radical scavenging enzymes like superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), and catalase (CAT) in the brain. In the present study, an attempt is made to formulate ginkgo phytosomes, to improve the solubility, permeability and drug release characteristics of Ginkgo.

MATERIALS AND METHODS

Materials: Ginkgo powder was obtained as gift sample from Mitushi Biopharma pvt ltd, Ahmedabad; Soy lecithin was procured from Sigma Aldrich, and solvents used in study like Dichloromethane and Hexane were of analytical grade.

Equipments: UV-Visible spectrophotometer (Shimadazu), Particle size analyzer (Microtrac Ltd), FTIR (Agilent Technologies).

Preparation of Ginkgo phytosomes:

Phytosomes are products formed by addition of a stoichiometric amount of phospholipid (phosphatidylcholine) to plant constituents in the presence of aprotic solvent. Solvent acts as a proton donor[6] for formation of phytosomes. Most commonly used phospholipids in the preparation of phytosomesare α-lyso phosphat-idylcholine and α-phosphatidy- lcholine. Phosphatidyl fraction of α-lyso phosphati dylcholine is lipophilic in nature which denotes the head of the compound and the hydrophilic choline fraction of α-lyso phosphatidylcholine acts as the tail of the compound. Choline portion of phosphatidy- lcholine bounds to the water soluble phytoconstituents. Lipid soluble portion combines with phosphatidyl part. The formed phyto phospholipid complex enhances the lipid solubility of drug. A chemical bond formed between hydrophilic and non-polar constituents plays a vital role for formation of minute spheres or tiny cells, which helpful the product from destruction by gastric microenvironment[8].

Formulation development of phytosomes involves addition of flavonoids, terpenoides, or aglycon glycosides in aprotic solvents like acetone, dichloro methane, dioxane, acetonitrile, DMF or DMSO[9]. As a result, phytosomes are precipitated out and seperated.

Preparation of Ginkgo Phytosome Complex

Rotary evaporator technique is utilized in the preparation of ginkgo phytosomal complex.

Rotary evaporation technique:

Accurately weighed quantity of Ginkgo (50mg) and soya lecithin were mixed in rotary round bottom flask. To the above preparation dichloromethane was added slowly. The flask was stirred for 1 hour at a temperature not more than 40^{o}C. A Thin film of the product was formed. To the formed product n-hexane was added and continuously stirred, until single layer of phospholipid is formed. 6.8 P^{H} Phosphate buffer was added to obtain final precipitate. The product is then placed in amber colored glass bottle11[1].

Preparation of Ginkgo phytosomes complex

Ginkgo phytosomal complex was formed by rotary evaporator method. Phospholipid to phytoconstituents ratios (1:0.5, 1:1, 1:1.5 1:2 and 1:2.5) were used, by varying the polymer concentration[12] (Table 1).

Table 1: Various Formulations codes of Ginkgo phytosomal complexes

Formulation Code	Ratio of Drug : Soy lecithin	Dichloromethane (ml)	n-hexane (ml)	6.8 P^{H} PBS (ml)
F-1	1:0.5	20	10	5
F-2	1:1	20	10	5
F-3	1:1.5	20	10	5
F-4	1:2	20	10	5
F-5	1:2.5	20	10	5

Evaluation of drug:

Ginkgo drug was evaluated for organoleptic properties such as color, drug solubility, and absorption maxima[13].

Drug solubility profile[14]:

Solubility was measured in various solvents such as DCM, hexane, ethyl acohol and pH 6.8 phosphate buffer.

Determining the wavelength:

Powdered Ginkgo was mixed with 3% Tween80 and P^{H}6.8 phosphate buffer (2: 8) [15]. Drug at concentration of 10µg/ ml was scanned UV spectrophotometer at 200 - 400nm wavelength range.

Compatibility:

The physicochemical interactions between drug and phospholipids in the formulation can be analyzed by FTIR spectroscopy and it is used to select appropriate chemically compatible excipients[16]. The purpose of FTIR spectroscopic study was to assess the interaction between the drug and the excipients.

Characteristics of Ginkgo Phytosomes

Measurement of Particle size:

Particle size determination of was carried with Microtrac analyzer. 300 µm of phytosomes were diluted with p^H 6.8 phosphate buffer and the diameter of the phytosome vesicles was measured.

Percentage yield:

Percentage yield of phytosomes was calculated using the formula[17] given below:

$$(\%)\ \text{Yield} = \frac{(\text{Practical yield}) \times 100}{(\text{Theoretical yield})}$$

Entrapment efficiency:

The product was centrifuged at 12000 rpm for 45mins using REMI centrifuge. Centrifuagtion helps to separate the unentrapped drug from the product [18]. Quantity of free drug was determined by measuring absorbance of preparation at 200 - 400 nm using UV-Visible spectrophotometer. The drug capture rate was determined using the formula:

$$\text{Entrapment efficiency } (\%) = \frac{\text{Amount of encapsulated medicament}}{\text{Amount of medicament added}} \times 100$$

Drug content:

Dr 10 mg of preparation was dissolved in 10 ml methanol. After suitable dilution, absorbance was determined at 400 nm and drug content was determined[19] using UV Visible spectrophotometer.

Scanning electron microscope (SEM):

SEM studies were performed to find out the surface morphology, size, and shape of the produced phytosomes[20]. The optimized phytosomes were placed under a scanning electron microscope and photographed.

Zeta potential (ZP) measurement:

Zeta potential was conducted to check physical stability of product. Higher Zeta potential values (electrostatic repulsion among the particles) indicates more stability. ±20 mV zeta potential indicates the higher physical stability of the phytosomal preparation. 1ml of the phytosomes were mixed with 10ml with water and transferred into cuvette and the zeta potential was determined using zetasizer.

In vitro drug release studies:

The invitro drug release was studied using open dialysis tube. The tube was filled with phytosomes (equivalent to 50mg Ginkgo). Dialysis tube was suspended in beaker, containing 250ml of P^{H}6.8 PBS. The temperature of the solution was kept at 37±0.5°C. With the help of magnetic stirrer the solution was stirred at 100 rpm and then 1ml phytosomal solution of was taken at specific intervals of time and equal volume of fresh phosphate buffer solution was added[21]. Sample was analyzed using UV-visible spectropho tometer. Drug release from drug alone and phytosomes were compared.

Release kinetics of Ginkgo phytosomes:

Release kinetics of the prepared phytosomes was carried out by fitting the data obtained from drug release studies in various kinetics model like (a) zero order model -cumulative percent drug released vs. time (b) first order model-Log cumulative percent drug retained vs. time (c) Higuchi model- Log cumulative percent drug released vs. square root of time) (d) Peppas model- Log of cumulative percent release Vs log time. The regression coefficient values were calculated. The release kinetics of optimized formulation is given in table number 3; the value of K (release rate constant) was calculated [22].

Stability studies:

The stability of optimized preparation was carried out as per ICH guidelines[23]. Optimized formulations were subjected at temperature ranges of 5^{0}C ± 3^{0}C, 30^{0}C ± 2^{0}C/65% ± 5% RH and 40^{0}C ± 2^{0}C/75% ± 5% for a period of 90 days and checked for content of drug and drug release profile[24].

RESULTS AND DISCUSSION:

Characterization of drug:

Organoleptic properties:

Drug was found to be colorless, soluble in pH 6.8 phosphate buffer, n-hexane and dichloromethane. The absorption maxima of 10 µg/ml of drug in phosphate buffer was found to be 260 nm as shown in figure 2.

Standard calibration curve of drug

The UV absorbance of varying concentrations drug solution in phosphate buffer solution pH 6.8 showed λ max at 260nm as shown in figure 2.

Figure 1: UV Spectra of Ginkgo

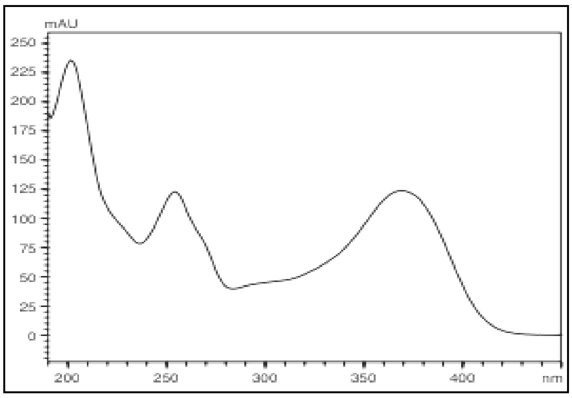
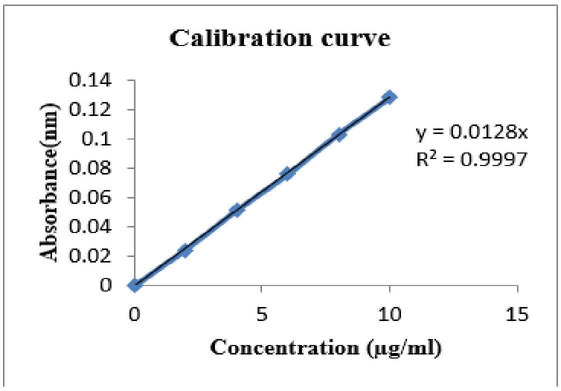


Figure 2: Standard calibration curve



Compatibility studies

Ginkgo and phospholipid was checked for compatibility by FTIR spectroscopy. No peaks were observed in the mixture indicating absence of interaction between the drug and excipient as shown in Figure 3 and 4.

Figure 3: FT-IR peak of Ginkgo

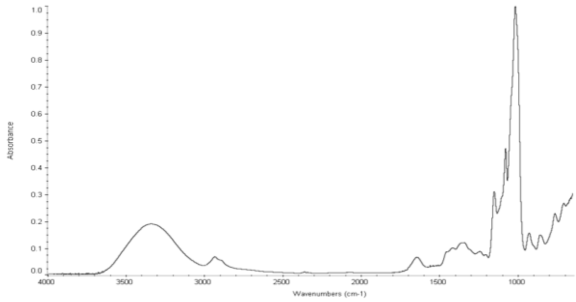


Figure 4: FTIR peak of Ginkgo-phospholipid

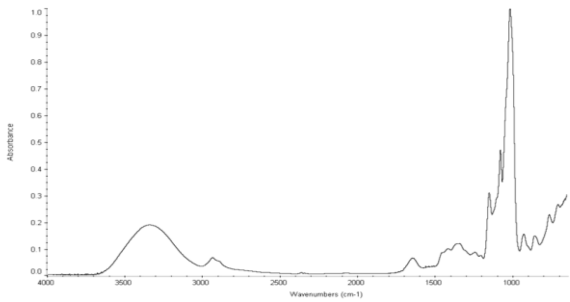
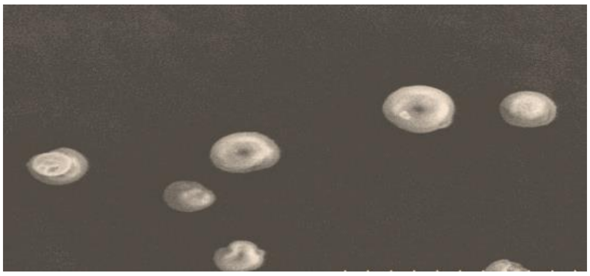


Figure 5: F-1 to F-5 Ginkgo phytosome

Surface morphology of Phytosome Complex:

The Surface morphology of vesicles was studied using scanning electron microscopy has shown presence of round vesicle as shown in figure 5 & 6.

Figure 6: Scanning electron microscopy image of formulation F-5



Particle size and Zeta potential:

Optimized batch of phytosomes were checked to find out their particle size and zeta potential. Particle size of optimized formulation F-4 was found to be 162.6nm and zeta potential value was found to be 29.4 mV indicating better stability.

Percentage yield, Drug content, Entrapment Efficiency of Ginkgo phytosomes:

Percentage yield of formulation F-4 that containing ginkgo and phospholipid in the ratio 1:2 showed highest % yield when compared to remaining formulations. Drug content of the optimized batch was found to be in between 80-94% as presented in table 2. The percentage yield was found in a range of 73-95 % and entrapment efficiency of the phytosome complexes was found in a range of 85-93%.

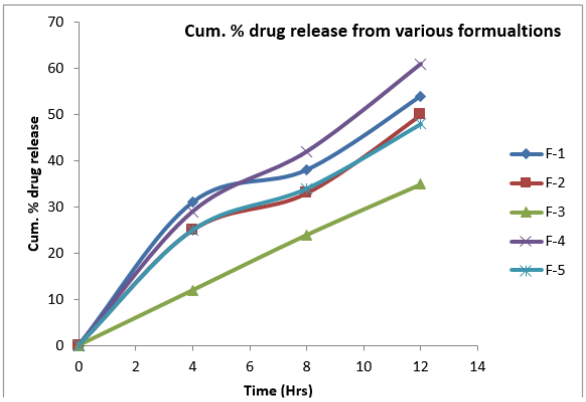
Table 2: Physicochemical characterization of Ginkgo phytosomes

Formulation Code	Percentage Yield (%)	Drug Content (%)	Entrapment Efficiency (%)
F-1	65.56	80.22	85.95
F-2	73.33	85.43	89.42
F-3	85.55	90.32	90.43
F-4	95.44	94.34	93.22
F-5	90.32	89.43	91.42

In-vitro drug release studies:

Phytosomes were loaded in a diffusion cell and receptor compartment was filled with phosphate buffer solution. The temperature was maintained at 37 ± 5^0C during experiment. Cumulative percentage drug release of formulation F-4 was found to be 89.54%. Invitro drug release of drug showed initial burst followed by slow release.

Figure 7: In-vitro drug release of Formulation F-4



Release Kinetics:

In-vitro drug release data of 5 formulations were fitted to kinetic models. Release profile was represented in table number 3. The "n" values were found to be more than 0.5. Release data follows non- Fickian diffusion method.

Table 3: Correlation Coefficients (r^2) for F-1 to F- 5 formulations

Formulation	Zero Order	First Order	Higuchi Plot	Peppas plot	
				r^2	n
F-1	0.817	0.885	0.896	0.934	0.783
F-2	0.826	0.921	0.934	0.940	0.801
F-3	0.847	0.951	0.954	0.926	0.823
F-4	0.915	0.998	0.993	0.919	0.912
F-5	0.832	0.864	0.876	0.922	0.876

Stability Studies

Optimized formulation F- 4 was stored at frozen temperature and room temperature for 120 days and drug release & percent drug content was determined. Optimized formulation F- 4 showed better drug release. There is no significant changes in drug content and drug release, when stored at frozen and at room temperature. The drug didn't undergo degradation during storage at various temperatures and humidity conditions.

Table 4: Stability data: Comparison of percentage drug content of formulation F-4 at various storage conditions

Storage condition	Drug content (%)
$5^0 \pm 3^0C$	92.35
30 ± 2^0C& 65±5% RH	93.52
40 ± 2^0C&75±5% RH	92.64

Figure 8: Percentage drug content of formulation F-4 at various storage conditions

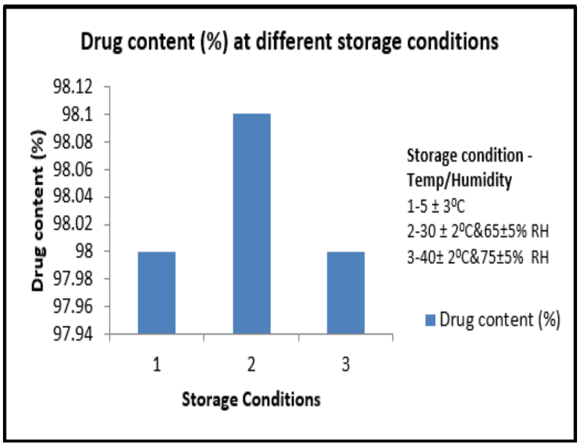
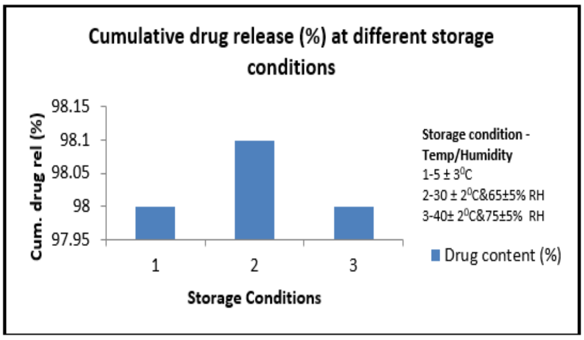


Table 5: Comparison of Cumulative % drug release from formulation F-4

Storage condition	Drug content (%)
5 ± 3^oC	98
30 ± 2^oC&65±5% RH	98.1
40 ± 2^oC&75±5% RH	98

Figure 9: Cumulative Percentage drug release formulation F-5 at various storage conditions



V. CONCLUSION:

Ginkgo phytosomes were manufactured using the Rotary evaporation process. The phytosomes obtained were globular in shape. The Phytosomes made using this process had a better particle size and good percent yield. Less particle size of phytosomes increases the drug absorption rate. The zeta potential was found to be greater than 20mV, indicating improved stability. With increasing polymer concentrations, the entrapment efficiency of prepared phytosomes has increased. Rotary evaporation method was found to be the best method for attaining high drug content and entrapment efficiency values. Formulation F-4 had shown low particle size, high zeta potential and in-vitro drug release. Stability tests were performed on the formulations and the findings revealed that the formulations had the highest drug content and better in-vitro release at all climatic conditions. Phytosomes prepared with drug: phospholipid ratio 1:2, i.e., Formulation F-4 produced round, separate vesicles of phytosomes which release the drug successfully for specific period of time.

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